

rylate and 1 mole of 2-dimethyl aminoethanol and 0.5 part of polypropylene glycol (a molecular weight: 2000) dissolved 10% of benzoin therein and pH of the obtained mixture was adjusted to 6. Then, 300 parts of the same acetoacetic decarboxylase aqueous solution as in Example 8 was added to the mixture and they were uniformly mixed.

This mixture was applied in the same manner as in Example 6 and a film of immobilized enzyme was obtained. The activity of the immobilized enzyme was then measured by Warburg constant volume manometer. As the result, it was understood that 1.2 times activity was obtained as compared with that of the immobilized enzyme which was obtained by using nonionic type photo-curable resin alone and in addition, operative stability of the immobilized enzyme was increased.

EXAMPLE 10

Photo-curable resin having nonionic hydrophilic groups was prepared by allowing to react an aqueous solution comprising 100 parts of 10% aqueous solution of polyvinyl alcohol (molecular weight: 70,000, rate of saponification: 90%), 2 parts of N-methylol acrylamide and 0.1 part of phosphoric acid for 5 hours at 60° C. and then neutralizing the reaction product with caustic soda. Further, photo-curable resin having ionic hydrophilic groups was prepared by allowing 2 moles of succinic anhydride to react with the reaction product which was obtained by allowing 1 mole of polypropylene glycol diglycidylether to react with 2 moles of acrylic acid.

Then, a uniform mixture was prepared from 40 parts of the former nonionic resin, 10 parts of the latter ionic resin, 10 parts of polypropylene glycol (molecular weight: 2000) having dissolved 10% of benzoin therein and 10 parts of acylase aqueous solution made by dissolving 0.5 g of acylase into 10 ml of phosphate buffer solution.

This mixture was applied in the same manner as in Example 6 except that irradiation was carried out for 3 minutes and a film of immobilized enzyme was obtained. This enzyme film was cut into plural pieces of 1 cm square. The 10 pieces of the cut film were then immersed into 10 ml of 50 mM N-acetyl-DL-methionine prepared by dissolving it in phosphate buffer solution at pH 7.5, and allowed to react for 30 minutes at 37° C. After the reaction, L-methionine yield was measured by the ninhydrin method. As the result, it was understood that the ratio of activity to that of native acylase was 70%.

COMPARATIVE EXAMPLE 1

A solution of 10 g of a dry hydroxyethyl methacrylate homopolymer (a molecular weight: about 8000) in 85 g of ethylene glycol monomethyl ether was prepared. A solution of 0.2 g of ammonium dichromate in 5 ml distilled water was then added and the solution was mixed during 5 minutes to produce a cross-linkable hydroxyethyl methacrylate polymer solution.

An aqueous solution of 1.0 g of glucose oxidase and 0.2 g of peroxidase in 300 ml of phosphate buffer solution at pH 7.0 was prepared, and 5 g of this solution was mixed with 45 g of the above polymer solution.

On a horizontal polyethylene film, a 0.2 mm thick layer of the mixture was cast. A flow of cold dry nitrogen was directed toward the surface of the film during 5 minutes. The film was then irradiated for 10 minutes with a 2 KW high pressure mercury lamp placed 10 cm above the surface to cross-link the polymer, thereby forming a film of immobilized enzyme. The activity of the immobilized enzyme was then measured by the

same manner as in Example 6. As the result, it was understood that the ratio of the activity to that of the immobilized enzyme of Example 6 was 10%.

What is claimed is:

1. A method for immobilizing enzymes or microbial cells which is characterized in the steps of:

uniformly mixing aqueous dispersion of enzymes or microbial cells with a monomer-free photo-curable resin having a number average molecular weight of 800 to 100,000, two or more photo-polymerizable ethylenically unsaturated groups per molecule and nonionic hydrophilic groups; and

irradiating actinic rays having a wave length of 2500 to 6000 Å to said mixture, whereby said enzymes or microbial cells are entrapped in said resin.

2. A method for immobilizing enzymes or microbial cells as claimed in claim 1 wherein said mixture additionally contains another monomer-free photo-curable resin having a number average molecular weight of 800 to 100,000, two or more photo-polymerizable ethylenically unsaturated groups per molecule and ionic hydrophilic groups, the ratio of the resin having ionic hydrophilic groups to the resin having nonionic hydrophilic groups being in the range of 1/99 to 50/50 by weight.

3. A method for immobilizing enzymes or microbial cells as claimed in claim 2, wherein said enzymes are urease, glucose oxidase, catalase, glucoamylase, glucose isomerase, invertase, acetoacetic decarboxylase, glucose oxidase-catalase, peroxidase, lactase, D-amino acid oxidase, α-galactosidase, aminoacylase, aspartase or penicillin amidase, and said microbial cells are the cells of *Lactobacillus bulgaricus*, *Aerobacter aerogenes*, *Bacillus subtilis*, *Azotobacter vinelandii* or *Proteus vulgaris*.

4. A method for immobilizing enzymes or microbial cells as claimed in claim 2, wherein said photo-curable resin having ionic hydrophilic groups is at least one member selected from the group consisting of salts of high acid value unsaturated polyesters, high acid value unsaturated epoxides, anionic unsaturated acrylic resins, cationic unsaturated acrylic resins, unsaturated polyamines and unsaturated carboxylated cellulose.

5. A method for immobilizing enzymes or microbial cells as claimed in claim 2, wherein said photo-curable resin having nonionic hydrophilic groups is at least one member selected from the group consisting of: polyesters made from polyethylene glycol and acrylic or methacrylic acid, urethanated adduct of the product made from polyisocyanate and polyethylene glycol with 2-hydroxyethyl acrylate or methacrylate, unsaturated cellulose, unsaturated polyvinyl alcohol and unsaturated polyamide.

6. A method for immobilizing enzymes or microbial cells as claimed in claim 2, wherein said ratio is in the range of 3/97 to 20/80 by weight.

7. A method for immobilizing enzymes or microbial cells as claimed in claim 2, wherein a photosensitizer is added to said mixture of photo-curable resins.

8. A method for immobilizing enzymes or microbial cells as claimed in claim 7, wherein said photosensitizer is at least one member selected from the group consisting of α-carbonyl alcohol, acyloin ether, α-substituted acyloin, naphthol, hydroxyanthracene, axoamide, uranyl nitrate, ferric chloride, mercaptan, disulfide, ascorbic acid and riboflavin.

9. A method for immobilizing enzymes or microbial cells as claimed in claim 2, wherein said number average molecular weight is 1,000 to 70,000.

10. A method for immobilizing enzymes or microbial cells as claimed in claim 4, wherein said high acid value is 40 to 200.

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